Chlamydia trachomatis infections in infants

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In recent years considerable progress has been made in understanding chlamydial infections. The spectrum of pediatric *Chlamydia trachomatis* infection includes neonatal inclusion conjunctivitis, infantile pneumonia, occasional respiratory or genital tract infections in older children and sexually transmitted diseases in adolescents. The role of maternal chlamydial infection in prematurity and in perinatal death is currently an area of active study. We outline the current knowledge of the biologic characteristics of *C. trachomatis*, the epidemiologic features of chlamydial infection, and the clinical aspects, diagnosis and treatment of neonatal chlamydial infections.

Depuis quelques années on comprend beaucoup mieux les chlamydioses. En pédiatrie les infections à *Chlamydia trachomatis* comprennent la conjonctivite à inclusions du nouveau-né, des pneumonies du nourrisson, quelques cas d'infection respiratoire ou génitale de la deuxième enfance, des infections à transmission sexuelle de l'adolescence. On étudie intensément le rôle que jouent les chlamydioses maternelles dans la prématurité et la mortalité périnatale. Nous esquissons ici l'état actuel des connaissances sur la biologie de *C. trachomatis*, l'épidémiologie des infections qu'elle cause, les aspects cliniques, diagnostiques et thérapeutiques des chlamydioses néonatales.

Chlamydiae are obligate intracellular bacteria that have been associated with a wide variety of human diseases.[1-4] They can be divided into two groups: *Chlamydia psittaci*, the causal agent of psittacosis, a zoonosis in which the natural life cycle occurs in birds and animals,[5] and *C. trachomatis*, the causal agent of trachoma. Trachoma is an important cause of blindness and affects approximately 500 million people, mainly in developing countries.[6,7] *C. trachomatis* has also been recognized as a pathogen of nongonococcal urethritis, salpingitis, endocervicitis, pelvic inflammatory disease (PID), inclusion conjunctivitis of neonates, infantile pneumonia and associated conditions.[8-10]

In this article we review recent progress in the investigation of *C. trachomatis* infections and the current literature on chlamydial infections in the neonatal period.

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Biologic characteristics of *Chlamydia*

The developmental cycle of chlamydiae is unique.[11-13] Infectious elementary bodies — small, dense, spherical bodies ranging from 200 to 400 nm in diameter — attach to host cells and are taken up by endocytosis. Within 6 to 8 hours they become noninfectious, metabolically active reticulate bodies 700 to 1000 nm in diameter (Fig. 1). During this time both elementary bodies and reticulate bodies can synthesize their own DNA, RNA and protein by using the host cell's energy-generating apparatus. Within 30 to 48 hours of entry, accumulation of glycogen within inclusion bodies can be demonstrated by iodine staining. Within 48 to 72 hours the host cells are destroyed and elementary bodies released to initiate a new cycle[14,15] (Fig. 2). The structure of the cell walls and membranes of chlamydiae is analogous to that of other gram-negative bacteria; however, the cell walls do not have a peptidoglycan component.

C. psittaci is a common pathogen of birds and domestic animals and can cause atypical pneumonia in adults and children.[16-18] There are many unidentified serologic variants (serovars) and biologic variants (biovars) of *C. psittaci*. Recently a new group of *C. psittaci*, known as TWAR, has been identified that has a cycle of human-to-human transmission without bird or animal intermediate hosts.[19-21] The name TWAR comes from the laboratory designation of the first two isolates

of this type, TW 183 (isolated from the eye of a child in Taiwan in 1965) and AR 39 (isolated from pharyngeal swabs of patients with acute respiratory disease in Seattle in 1983). The TWAR agent is associated with cases of human pneumonia and acute respiratory disease in adults and teenagers.[21] A recent study has shown that the organism plays an important role not only in pneumonia and bronchitis but also in pharyngitis and sinusitis.[19] However, the role of the TWAR agent in cases of illness in children, particularly during the neonatal period, is uncertain.

The other species of *Chlamydia*, *C. trachomatis*, has a well-defined natural life cycle in humans. There are three biovars of *C. trachomatis*: the causal agent of mouse pneumonitis (MoPn), the agent of lymphogranuloma venereum (LGV) and the agent of trachoma (human oculogenital disease).[22,23] There are 12 serovars of the trachoma biovar (A to K) and 3 serovars of the LGV biovar (L_1, L_2 and L_3). Inclusion conjunctivitis and infantile pneumonia are associated with trachoma serovars D through K. Chlamydiae have a lipopolysaccharide genus-specific antigen closely related to the ketodeoxyoctanoic acid–lipid A core glycolipid of gram-negative bacteria. This antigen is heat stable and is identified by a complement-fixation reaction. A protein that appears to be the major structural component is the predominant site of immunoreactivity, defining species specificity and subspecies or serovars. The species-specific antigens are detected by indirect hemagglutination, immunodiffusion, crossed immunoelectrophoresis and the microimmunofluorescence test.[24] Monoclonal antibodies have also been used to determine serotypes of *C. trachomatis* in clinical isolates. Double-stranded DNA is completely homologous between the trachoma and LGV serovars, but there is only 30% to 60% homology between the mouse pneumonitis serovars and the trachoma and LGV serovars. Less than 10% of DNA is homologous between *C. psittaci* and *C. trachomatis*. Diagnostic tests based on the use of monoclonal antibodies and enzyme-linked immunosorbent assays (ELISAs) are widely used in screening for *C. trachomatis* infections.

Epidemiologic features of *C. trachomatis* infections

Trachoma is a chronic form of keratoconjunctivitis with a marked follicular reaction and papillary hypertrophy of the conjunctiva. The disease was once common in virtually all countries, but improved living standards have resulted in its disappearance from most communities in Europe, North America and Japan. It is still the leading cause of blindness in a large area extending from north and sub-Saharan Africa through the Middle East and into the Indian subcontinent.[25-27]

C. trachomatis has recently been recognized as an important cause of sexually transmitted disease (STD), including nongonococcal urethritis and epididymitis in men and cervicitis and PID in women.[28,29] It is estimated that the organism causes approximately 3 million cases of STD each year in the United States.[7] Chlamydial urethritis is 2.5 times more common than gonococcal urethritis in men, and approximately 50% of all cases of nongonococcal urethritis are caused by *C. trachomatis*.[14,30] At STD clinics chlamydial urethritis is only about one-third as common in homosexuals as in heterosexuals.[30] In women *C. trachomatis* is a common cause of cervicitis and the urethral syndrome. It also causes half of the cases of PID diagnosed each year.[31] Being young, being of lower socioeconomic status and having several sexual

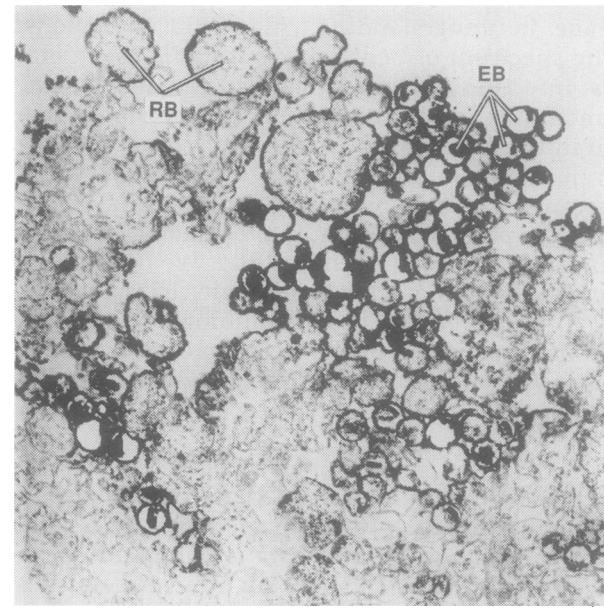

Fig. 1 — Electron micrograph (×40 000, reduced 30%) of microcolony of L_2 strain of *Chlamydia trachomatis* in cytoplasm of McCoy cell. Elementary bodies (EB) and reticulate bodies (RB), stained with monoclonal antibodies, are clearly distinguishable.

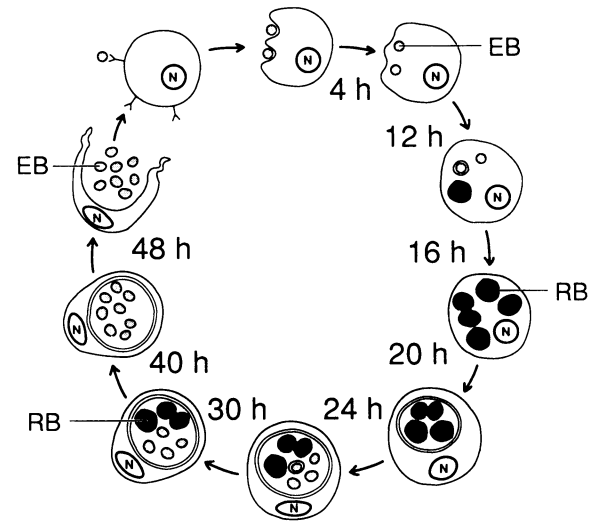

Fig. 2 — Growth cycle of chlamydiae.

partners are all associated with increased rates of *C. trachomatis* infection in women.[31] Several investigators have reported that 2% to 20% of pregnant women may have *Chlamydia* in their endocervix.[32-34] Pregnant women who carry *C. trachomatis* in their genital tract may suffer from a generalized disturbance of immunoregulation.[8,35,36] Responsiveness of their lymphocytes to the organism, other microbial agents and T-cell lectins is suppressed during pregnancy.[37] The clinical relevance of this finding remains unknown.

It has been suggested that *C. trachomatis* infection in pregnant women may be related to premature labour and delivery and to perinatal death.[38-41] Transmission of the organism from mothers to their infants generally occurs at the time of delivery with passage of the infant through the infected cervix, but the possibility of intrauterine infection has been reported in infants born by cesarean section.[42,43] Inclusion conjunctivitis and a distinctive afebrile form of pneumonia may develop during the first 6 months of life in neonates exposed to *C. trachomatis*.[44-47] Inclusion conjunctivitis develops in 18% to 50% of newborns born to infected women, and infantile pneumonia develops in 3% to 20%.[31-33,48]

Studies showing appreciably higher rates of seropositivity in children aged 7 to 15 years than in infants or pre-school-age children suggest the possibility of other nonoculogenital chlamydial infections in children that are as yet undefined.[49-51] However, *C. trachomatis* is rarely found in the oropharynx of adolescents and school-age children and is not a common cause of pharyngitis.[52,53]

Neonatal inclusion conjunctivitis

Symptoms of neonatal inclusion conjunctivitis are usually present 5 to 12 days after delivery,[54-56] in contrast to those of gonococcal conjunctivitis, which appear 3 to 5 days after birth. In several cases of premature rupture of the membranes conjunctivitis due to *Chlamydia* appeared as early as the day of birth.[48] The clinical features range from almost asymptomatic infection to severe purulent conjunctivitis. Unilateral eyelid swelling is also a common presentation, and pseudomembranes are occasionally seen. Although ophthalmic discharge often appears, it is seldom mucopurulent. Papillary hypertrophy of the upper tarsal plate is characteristic. Clinical signs of trachoma, including keratoderma, scarring of the conjunctiva and formation of pannus, are rare.[57] Classic follicular conjunctivitis does not occur during the first month or two of life because of an absence of lymphoid cells in the ocular tissues of neonates. Infiltration of subepithelial tissues of the conjunctiva by polymorphonuclear exudate, followed by macrophages, lymphocytes and then plasma cells, is evident on pathological examination, and inclusion-body-laden epithelial cells may be seen.

If untreated, active disease may persist for 3 to 12 months or may clear spontaneously. Occasionally ocular carriage of *C. trachomatis* persists for as long as 2 years after birth.[58] It has been suggested that trachoma may be a result of recurrent ophthalmic infection and inflammation due to several different serovars of *C. trachomatis*.[59-62]

Pulmonary diseases of newborns

In 1975 Schachter and colleagues[45] reported a case of chlamydial pneumonia that developed shortly after inclusion conjunctivitis. Other investigators subsequently reported a relation between *C. trachomatis* infection and infantile pneumonia.[63-66] The clinical characteristics of chlamydial pneumonia in infancy are now well documented.[67] Infected infants show symptoms at about 4 to 11 weeks of age. Characteristic symptoms and signs include persistent staccato cough, wheezing, rales, tachypnea and, sometimes, tympanic membrane involvement. Tachypnea and cough are often preceded by 1 to 2 weeks of rhinorrhea. More than 95% of infants are afebrile. Approximately 50% have concurrent conjunctivitis or a history of conjunctivitis.

Characteristic laboratory findings are leukocytosis with eosinophilia (eosinophil count 0.4×10^9/L or greater) and elevated levels of serum IgG (5.00 g/L or more) and IgM (1.10 g/L or more). Hyperinflation and diffuse interstitial pulmonary infiltrates are seen on chest roentgenograms.[67] Other pathogens, including respiratory syncytial virus, cytomegalovirus, *Ureaplasma urealyticum* and *Bordetella pertussis*, may cause forms of pneumonia of similar clinical presentation.[66] Several investigators have reported that immune disregulation may contribute to the development of chlamydial pneumonia.[68] Infants with chlamydial pneumonia often have severe hyperimmunoglobulinemia and an increased number of B lymphocytes and plasma cells.[68] The B-cell activation seems to be polyclonal: little of the hypersecreted IgG, IgM and IgA is directed against *C. trachomatis*. Similar abnormalities in serum immunoglobulins have been reported in cases of LGV.[69,70]

Chlamydial infection and pregnancy

Intrauterine infection with *C. trachomatis* may occur. Chorioamnionitis is a frequent finding in prematurity and in respiratory insufficiency in premature babies and may be attributable to intrauterine infection.[71]

Widespread concern has arisen in recent years that chronic respiratory disease in infancy and childhood may result from chlamydial pneumonia.[72] Both bronchopulmonary dysplasia (BPD) and Wilson–Mikity syndrome are chronic lung diseases of premature infants.[73,74] BPD usually occurs after treatment of respiratory distress syndrome with mechanical ventilation, but the relative

importance of oxygen, barotrauma and endotracheal intubation in the development of BPD is controversial.[75] The cause of Wilson–Mikity syndrome is unknown. Some infants are born with severe respiratory distress, which resembles respiratory distress syndrome and requires oxygen. Clinical, radiologic and pathological findings similar to those in Wilson–Mikity syndrome have been demonstrated in infants with BPD.[74] The fact that neonates who present with typical features of Wilson–Mikity syndrome also manifest greatly elevated serum IgM levels at birth suggests that intrauterine infection may play a role[76,77] (Table I). The data of Table I also suggest that some cases of chronic lung disease may be caused by intrauterine infection. A relation between maternal chlamydial infection and prematurity has recently been reported.[43] An association between *C. trachomatis* infection and chronic lung disease in premature infants has also been suggested.[78–80]

Other diseases of newborns caused by *C. trachomatis*

Rectal and vaginal sites in exposed infants may be colonized with *C. trachomatis*. Diseases due to these infections are not well described. Gastrointestinal symptoms have generally not been associated with rectal carriage of *Chlamydia*.[44]

C. trachomatis has been isolated from middle-ear fluid in infants with pneumonitis and otitis media.[81] On the basis of serologic studies it has been suggested that the organism may cause myocarditis in children.[82] Although genital tract infections due to *C. trachomatis* in sexually active adolescents are well known,[83–86] vaginal colonization with the organism has not been associated with disease in infancy. Genital tract infections due to *C. trachomatis* in newborns are rare except in cases of sexual abuse.[87] Chlamydial meningoencephalitis has been reported in adults but not in newborns.[88]

Diagnosis of *C. trachomatis* infection

The demonstration of characteristic intracytoplasmic inclusion bodies of *C. trachomatis* in clinical specimens by means of Giemsa, iodine or Papanicolaou staining is routine procedure for the diagnosis of chlamydial infection.[89] These methods are very specific and reliable when properly carried out and when the clinical samples have been properly collected. Scrapings of the upper or lower conjunctiva may be taken in infants suspected of having inclusion conjunctivitis. For infants with pneumonia, swabs may be collected from the posterior nasopharynx; nasopharyngeal aspirates are also acceptable.[46,47]

Isolation of *C. trachomatis* by means of tissue culture with McCoy or HeLa cells treated with cycloheximide or diethylaminoethyl–dextran should also be done.[90–93] For this purpose nasopharyngeal aspirates and conjunctival swabs are obtained from infants with pneumonia and conjunctivitis respectively. Swabs with cotton, rayon or Dacron tips and a plastic or aluminum shaft should be used. The specimens should be placed in a special transport broth that includes cell culture growth medium, antibiotics and fetal calf serum; commonly used for this purpose are 2SP medium (sucrose, phosphate buffer, fetal calf serum and antibiotics) and a sucrose–phosphate glutamate (SPG) medium. The specimens should be refrigerated and processed within 24 hours or frozen at −60°C until processed. The McCoy or HeLa cells are usually grown on coverslips, onto which clinical specimens are centrifuged. After an appropriate incubation period the monolayers are stained with fluorescein-conjugated antibodies to *C. trachomatis*, enzyme-conjugated monoclonal antibodies, iodine or the Giemsa method. If characteristic intracytoplasmic inclusion bodies are detected, *C. trachomatis* is considered present. In some cases blind subculture of inoculated cell cultures may improve the isolation rate.

An immunofluorescence assay (MicroTrak, Syva Co., Palo Alto, California) using fluorescein-conjugated monoclonal antibodies is available for rapid clinical diagnosis.[94–100] For this purpose the nasopharynx of infants with pneumonia and the conjunctiva and nasopharynx of infants with conjunctivitis are scraped with a Dacron-tipped swab. The swabs are then rolled onto specially prepared antigen-impregnated slides. The specimens are air dried, fixed and stained directly with fluorescein-conjugated monoclonal antibodies for the detection of elementary bodies. The sensitivity and specificity of this technique in the assessment of newborns

Table I — Clinical features of premature infants with or without chronic lung disease[76]

Feature	Infants with chronic lung disease (n = 22)	Infants without chronic lung disease (n = 40)
	Mean (and standard error)	
Gestational age, wk	27.6 (5.3)	29.0 (2.7)
Birth weight, g	1094 (344)	1050 (251)
Level of IgG in cord serum, g/L	4.37 (176)	4.51 (1.71)
Level of IgM in cord serum, g/L	0.45 (0.46)	0.16 (0.19)
	No. of cases	
Elevated serum IgM level (≥ 0.20 g/L) at birth	12	3
Premature rupture of the membranes	11	13
Prolonged rupture of the membranes (> 24 h)	8	7

with conjunctivitis have been reported to be 100% and 94% respectively compared with tissue culture.[94] In contrast, a sensitivity of only 85% and a specificity of 75% have been reported with nasopharyngeal specimens.[94] Thus, the sensitivity and specificity of the test may vary with the test site and population evaluated. Cross-reacting bacteria may give confusing results when oral sites are sampled; for example, false-positive findings may result from the ability of some of the monoclonal antibodies to bind nonspecifically to protein A of *Staphylococcus*. This can be a serious problem when the test is carried out by inexperienced personnel. In general, however, the immunofluorescence assay is a relatively rapid and reliable technique. It is probably at least as sensitive and specific as the ELISA in the hands of trained personnel and is better suited for laboratories that handle small numbers of samples.[99] The test is suitable as a substitute for culture in screening adolescent populations with a moderate to high prevalence of genital infection and in confirming a diagnosis of symptomatic neonatal conjunctivitis.[101]

An ELISA (Chlamydiazyme, Abbott Laboratories, North Chicago) is also available for detecting chlamydial antigens in clinical specimens.[102] The sensitivity and specificity of this procedure have been reported to be 98% and 94% respectively in the detection of *C. trachomatis* in conjunctival specimens from infants with conjunctivitis.[103] In nasopharyngeal infections the rates were 87% and 92% respectively. For purposes of the ELISA, specimens are obtained with Dacron or wire swabs (such as STD-PEN) (Abbott Laboratories) from the conjunctiva and nasopharynx of infants with conjunctivitis or pneumonia. These swabs can also be used for collecting urethral samples. The swabs are immediately immersed in a specially provided storage solution and are transported to the laboratory. Specimens for ELISA can be refrigerated at 2°C to 8°C for up to 5 days. The test is semiautomated, and although special equipment is needed it is suitable for processing large numbers of specimens. The disadvantages are the longer time required to obtain results and an inability to evaluate the adequacy of the specimen before testing. Again, false-positive results, especially from nasopharyngeal specimens, owing to cross-reacting bacteria, have been reported, and caution should be used in interpreting the results.[103] One potential source of problems is that since a polyclonal antibody is used in the ELISA, cross-reactions can occur with a wide variety of other bacteria commonly found in the anogenital region. For this reason, genital specimens should be obtained exclusively from the urethra and cervix.

Neither the immunofluorescence assay nor the ELISA is sensitive enough to reproducibly detect evidence of *Chlamydia* in specimens from asymptomatic male patients.[101] Furthermore, these rapid methods should not be relied on in cases of sexual abuse. These tests are not intended to replace "gold standard" methods (i.e., tissue culture performed by competent laboratories).[101]

Serologic diagnosis of *C. trachomatis* infection is usually inefficient except in cases of infantile pneumonia, in which the detection of serum IgM antibodies to the organism may be useful.[104,105] One of the most reliable methods of detecting antibodies to *C. trachomatis* is a microimmunofluorescence test.[24,106] However, this method has not been readily available to most diagnostic laboratories because of the need for numerous test antigens of different kinds of elementary bodies. Similar immunofluorescence tests using reticulate bodies or infected cells as test antigens have also been described.[107-110] Recently antichlamydial antibodies have been detected by means of the ELISA or a refined enzyme immunoassay.[111-114] The ELISA method is relatively inexpensive and is highly reproducible. A number of commercially available ELISA kits are now available for this purpose, and there seems little doubt that this method will be preferred for the foreseeable future. These assays have been used to detect antibodies in patients with psittacosis and LGV as well as in newborns and infants with ocular and respiratory chlamydial infections. Refined enzyme immunoassays for serum IgG, IgA and IgM against various serovars of *C. trachomatis* have been reported to have high sensitivity and specificity.[111-114]

Molecular biologic techniques have also been used in the diagnosis of *C. trachomatis* infection.[115] *C. trachomatis* nucleic acid may be detectable by in-situ hybridization in both infected tissue culture cells and in clinical specimens. In-situ DNA hybridization methods may be particularly useful for diagnosis in cases in which small amounts of tissue are obtained by biopsy or autopsy.

Therapy and prevention of *C. trachomatis* infections

Silver nitrate, which was originally used for preventing ophthalmic infections due to *Neisseria gonorrhoeae*, is not effective against *C. trachomatis*.[116,117] Erythromycin (0.5%) or tetracycline (1%) ointment may prevent ophthalmic *C. trachomatis* infection but not nasopharyngeal colonization or pneumonia.[118] Oral erythromycin therapy (50 mg/kg per day in four divided doses administered for 14 days) has been recommended for both neonatal inclusion conjunctivitis and chlamydial pneumonia.[119] Sulfonamide antimicrobials are an acceptable alternative in infants at least 3 to 4 weeks old.[120]

One of the most important ways to prevent neonatal chlamydial infections is by appropriate screening and treatment of infected women and their sexual partners before delivery.[121] Erythromycin is nontoxic for fetuses and can therefore be administered to pregnant women.[122] Preferred forms of treatment include erythromycin base or stearate (500 mg given orally four times a day for 7

days) and erythromycin ethylsuccinate (800 mg given orally four times a day for 7 days).[122] In most cases erythromycin is effective in the treatment of cervical infections during pregnancy and in the prevention of vertical transmission to newborns. In cases of gastrointestinal discomfort, amoxicillin, which has fewer side effects and better patient compliance than erythromycin, may be considered as an alternative.[123] A recent preliminary study showed that orally administered amoxicillin (500 mg three times a day for 7 days) is as effective as erythromycin in the treatment of chlamydial infections.[123]

Conclusion

C. trachomatis has been recognized as an important pathogen of STD in adults and teenagers, of neonatal inclusion conjunctivitis and of infantile pneumonia. The clinical features of neonatal inclusion conjunctivitis and infantile pneumonia have been well documented.

However, the role of *C. trachomatis* in childhood infections remains largely speculative. Other, still undefined nonoculogenital pediatric infections due to the organism may exist.[49-51] The role of maternal *C. trachomatis* infection in prematurity and in perinatal death is currently an area of active study. Further study, including investigation of the pathological role of *C. trachomatis* in the perinatal period, is needed.

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